SYMPOSIUM ON RADIATION EFFECTS ON CELLS AND BACTERIA[1]

ALBERT KELNER

Brandeis University, Waltham, Massachusetts

W. DEXTER BELLAMY

Research Laboratory, General Electric Company, Schenectady, New York

G. E. STAPLETON

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

MAX R. ZELLE

Laboratory of Bacteriology, Cornell University, Ithaca, New York

INTRODUCTION

ALBERT KELNER

Radiobiology is an exciting field today. Old theories are found inadequate; new ones push forward for recognition. Of all the cross-disciplines, radiobiology is one of the broadest. At a meeting for radiobiology, there mingle physicians, embryologists, physiologists, biophysicists, biochemists, physical chemists, geneticists, bacteriologists, and virologists. All must learn each other's language. The researcher continually meets viewpoints from outside his own specialty. And ever present is the feeling that the solution of the problems of radiobiology may be a life and death matter for the human race, threatened with disaster from the increasing radiation of the atomic age.

Among the recent developments which have brought radiobiology to its present interesting, if somewhat confused, state are: (a) The target theory, which for many years dominated thinking in this science, has proven unsatisfactory. It simply does not explain enough. But no equally unifying theory has yet risen to replace the target theory, which still remains the most useful single concept we have. (b) Radiation effects have proven to be reversible (or preventable) to a degree not previously suspected. In addition to photoreactivation, there are also heat, catalase, and nutritional reactivations. Others are likely to be discovered. (c) The indirect action of radiations has now been shown to be a most important part of radiation mechanisms. The most notable examples are the reactions underlying the oxygen effect in ionizing radiations, as described by Stapleton in this symposium. (d) A large number of chemicals have been discovered which have effects similar to radiation. No longer are radiations the sole inducers of mutation. In addition, exact knowledge of an almost bewildering variety of end effects of radiations has accumulated. To name only a few: lethality, gene mutation, chromosomal aberrations, and various types of growth inhibition. Then there are such less understood but significant effects as the induction of lysogenesis in bacteria.

What is the relation between radiations and radiomimetic chemicals? What is the link between all the end effects? The scientist can attack such an apparently indigestible mass of data in two ways. He can look for all possible differences between various types of radiations and their effects and try to organize the data in this fashion. Or he can seek some common denominator and unify the material with some generalization. Both methods are sound and serve as a check upon each other.

Perhaps a way to simplify the discussion is to consider the one feature characteristic of almost all radiation effects—the delay between the initial inciting agent, and the first appearance of an end effect, be it mutation or death of the cell. Zelle in this symposium ably discusses some of the theories for this delay, especially in connection with the mutagenic action of radiations.

We can divide the events in an irradiated cell into three periods: a beginning, a middle, and an

[1] This symposium was presented before the Fifty-third Annual Meeting of the Society of American Bacteriologists at San Francisco, California, on August 11, 1953. Professor Carl Lamanna, Vice-chairman of the General Division, organized the symposium, and Albert Kelner was the convenor and editor of these papers.

end. The beginning period includes the instant of irradiation, the primary reactions, ionizations or excitations; the middle, all that time after the primary reactions and before the end effects are measurable. Its duration will vary with such factors as temperature and menstruum of the cells, and with the particular end effect observed. Finally there is the end period, in which an alteration or lesion of the cell manifests itself.

Bellamy, in this symposium, describes some of the basic features of the beginning events. These can be studied with relative exactitude. The beginning period will probably be understood long before the other two. It is the special province of the radiochemist and biophysicist although the biologist can help by determining the significance of the cellular site where the reactions occur. Protective agents which work only if present during the instant of radiation probably influence the beginning reactions. These primary reactions, important as they are, are only a part, perhaps a small part, of the story.

The middle period is unexplored territory. During this period the reactions leading to the end effects occur. During this stage the cell is in an unusual physiological state, characterized by an inhibition of certain syntheses, desoxyribose nucleic acid and adaptive enzymes being the most interesting, and, equally significant, an absence of almost all other effects. These events are undoubtedly a reflection of some specific alterations in the functioning of the nucleus, while cytoplasmic functions are untouched.

This is easy to say, but what precisely are the functions of the nucleus? The problems of radiobiology become part of a bigger problem, the relationships of nucleus and cytoplasm in the life of the cell.

The theory held by the writer is that all irradiated cells (and perhaps even cells treated with radiomimetic chemicals, too) go through a similar physiological "middle" period before manifesting the end effects. The question whether these middle reactions are the cause of the end effects, or coincidental results of some other common reactions, is unanswered.

Whatever the situation, the middle period certainly has to be understood before we can hope for any real understanding in radiobiology. The solution requires the combined efforts of the cellular physiologist, the biochemist, geneticist, and microbiologist. Such coordination of efforts is at once the difficulty and the thrilling challenge of radiobiology.

PART 1. RADIATION EFFECTS ON SOME ISOLATED CELL CONSTITUENTS

W. D. BELLAMY

The over-all problem confronting the radiobiologist is: how can a dose of ionizing radiation that affects only one molecule in 10^7 or less in the cell, kill a cell or cause it to mutate? The limited knowledge of the physical chemistry of semiliquids and complex molecules makes impossible complete interpretation of events in the complex, highly organized cell. How then can we hope to understand *the* biological effects of radiation? The answer is that we probably cannot, but what we can understand is *some* of the biological effects of radiation.

Of the several approaches to the problem, one of the more obvious is a study of the radiation effects on isolated components of cells. The knowledge gained from the action of radiation of the individual components can be extrapolated, it is hoped, back to the original cell. A valid criticism of this approach is that cell components interact with each other and that the whole may be greater than the sum of its parts. Critical irradiation effect(s) may possibly never be observed in isolated cell components. Although this approach is limited, use of it may teach us a great deal about the types of reactions that have been found to occur in irradiated cells.

In this section several examples of the types of reactions that have been found to occur in irradiated cell components will be given. These examples are both from the literature and from our experimental work. Most of our experimental results were obtained with high-velocity electrons (1, 2).

A one-million electron volts (Mev) resonant transformer type X-ray unit was modified to a cathode-ray source by replacement of the tungsten target on the accelerating tube by a thin stainless steel window. Operating at 800 kilovolt (kv) peak and 100 microamperes beam current, a dose was accumulated in a sample 10 cm from the window at the rate of 140,000 roentgen equivalent physical (rep) per second.

It is not the purpose of this paper to discuss at length the effect of all the different types of ionizing radiation. I will limit my discussion to high-velocity electrons and to X-rays which have similar specific ionizations. The energy of

the high-velocity particle is dissipated or absorbed in biological materials by excitation and ionization. The principal means of energy dissipation by ionizing radiation on its passage through matter is by ejection of electrons from the atoms through which it passes. The atom that has been ionized is left with a positive charge. The ejected electron eventually becomes attached to another atom and makes a negative ion. The energy of ionization averages about 32 electron volts which is far in excess of the energy of chemical bonds, which is less than 10 electron volts. It is the chemical action of the ions produced, particularly the positive ions, that is of importance in the biological effects of radiation. For general discussions of the mechanism of absorption of ionizing radiations the reader should consult Lea (3), Morrison (4), and Fano (5).

Excitation occurs when the energy imparted to an electron is insufficient to eject it from the atom but is enough to raise it to a higher energy state. Because of the low quantum yield of ultraviolet light in most biological materials, it is generally considered that excitation contributes a very minor part of the biological effects of ionizing radiation. Unlike visible or ultraviolet (UV) light, the absorption of ionizing radiation is essentially independent of the chemical combination of the atoms but is dependent upon the atomic number.

A large fraction of the cell is water, and the effects of ionizing radiation on water are of primary interest. In spite of much work over a period of 25 years (6, 7), the absorption processes in a material as simple as water are not completely understood. It has been well established that appreciable quantities of H_2O_2 are produced by X-ray or γ-rays only in the presence of dissolved oxygen. Densely ionizing particles such as α-rays will produce H_2O_2 from pure water. The active radicals HO and HO_2 are thought to be responsible for most of the indirect effects on biological materials. In the presence of oxygen, H_2O_2 becomes an important factor.

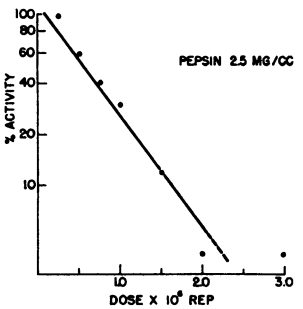


Figure 1. The inactivation of pepsin with 800 kilovolts (peak) electrons. Irradiated at room temperature in air. Pepsin concentration, 2.5 mg per ml. The dose was accumulated at the rate of 143,000 roentgen equivalent physical (rep) per second. (From Bellamy and Lawton, Nucleonics, **12**, 54–57, 1954.)

If an ionization occurs within the biological molecule, it is called a direct effect, and if the energy is absorbed by ionization in the solvent and the ionized or activated solvent molecules react with the solute, it is called an indirect effect. The so-called target theory is based upon the direct effect and the identification of the target with a definite biochemical entity. Lea (3) developed and expanded the target theory, and Pollard and co-workers (8) have skillfully exploited the theory for the examination of internal structures of enzymes, antibodies, viruses, bacteria and bacterial spores.

The study of the biochemical effects of radiation becomes a study of what factors affect these two methods of energy absorption. There are few things one can do to a biological system that will not affect one or the other of these processes.

Several examples can be given of both direct and indirect effects. The inactivation of pepsin by high-velocity electrons will illustrate these effects. Figure 1 shows that the inactivation of pepsin in dilute solution by high-velocity electrons is a first order reaction. The simplest way to report this is as the mean inactivation dose or the dose necessary to reduce the activity to 37 per cent, or 1/e, of the original activity. In terms of the target theory this is the dose necessary to score an average of one effective hit per target volume. Table 1 shows that the 37 per cent dose increases with concentration, while the dose necessary to inactivate a unit of pepsin remains fairly constant. The values greater than 5 mg per ml are for suspensions of the enzyme rather than for solutions (9). The results agree with those of Dale (10) on the X-ray inactivation of carboxypeptidase but disagree with those of McDonald (11) for the X-ray inactivation of trypsin.

It seems logical that anything that will affect the efficiency of transfer of energy from the solvent to the solute will affect the ionic yield, *i.e.*, the destruction of pepsin. One method of reducing the transfer efficiency is to immobilize

the solvent ions by freezing. Figure 2 illustrates the results of this procedure. The inactivation increases from 10 per cent to 90 per cent when the solvent ions become mobile at the change in phase. The inactivation dose for frozen pepsin is only slightly less than that for dry pepsin, indicating that most of the effect on frozen pepsin solution is due to direct effect (9).

Another method for decreasing the efficiency of energy transfer from the solvent to the solute is to add so-called protectors, *i.e.*, other solutes. Compounds which react more readily with the solvents ions than do the protein molecules are effective protectors (*e.g.*, thiourea, cystine, sodium nitrite, glutathione, hydrosulfite). Many other compounds are less reactive than the protein molecules and are relatively ineffective as protectors. Thus Dale (10) has reported that thiourea is about 20 times as effective as glucose in the protection of carboxypeptidase against X-rays.

Such protectors can be effective against the indirect action and can have little effect on the direct action. As shown in figure 1 the fraction of inactivation due to direct action increases with concentration. The problem of determining the relative amounts of inactivation due to these two methods has no ready solution. A few examples can be given of the inactivation of enzymes in natural products. Each individual enzyme represents only a small fraction of the total solutes, and therefore one would expect considerable protection from indirect action. The inactivation dose for catalase in crushed potato is about 5×10^6 rep whereas a pure catalase solution of 1 mg per ml has an inactivation dose of about 25,000 rep. Although other factors such as peroxidases are involved in the crushed potato, the difference in inactivation dose is a measure of the protection offered by the other constituents of the cells. The inactivation of tyrosinase (phenol-oxidase) in crushed apples is another example of the radiation protection that cell components provide each other (9). The inactivation dose for tyrosinase in apples, when irradiated at 25 C, is 5 to 7×10^6 rep. If the apple is irradiated at −78 C, the inactivation dose increases to about 10×10^6 rep. This slight increase should be compared with figure 1 and again indicates that most of the inactivation of tyrosinase must be due to direct action.

Within wide limits, the dose rate is not important in whole biological systems. It appears that reactions which are both initiated and terminated by free radical mechanisms are rate sensitive; an example of this is the polymerization of tetraethylene glycol dimethacrylate (TEGMA). Schmitz and Lawton (12) showed that the rate of dose accumulation influences profoundly the quality of the polymer. If the dose rate is high, the radicals terminate the chain before large polymers can be formed; a low dose rate favors longer chains. Mead (13) found that the oxidation of linoleic acid is rate sensitive. This unsaturated fatty acid autooxidizes by a free radical mechanism (14).

We have found no essential difference in the survival of *Escherichia coli*, strain B/r, to X-rays

TABLE 1

Relation between the concentration of pepsin, D_0 dose, and ionic yield

Concentration	$D_0 \times 10^6$ (37% D)	Ionic Yield
mg per ml		
0.1	0.042	0.0142
0.5	0.17	0.0178
1.0	0.34	0.0178
2.5	0.85	0.0178
5.0	1.3	0.0234
10.0	2.6	0.0234
20.0	3.4	0.0356
40.0	6.4	0.0379
Dry	30.0	0.22

The pepsin solutions and suspensions were irradiated neutral at room temperature and assayed in 0.06 N HCl.

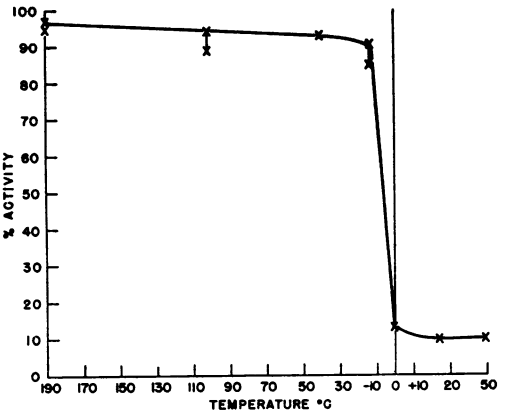
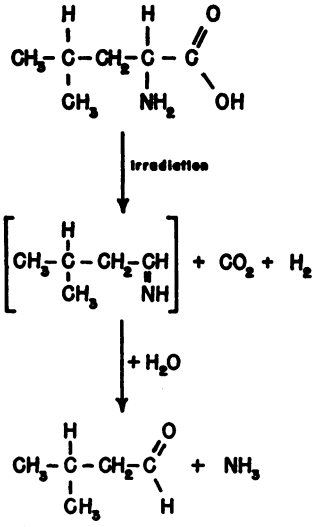


Figure 2. Effect of temperature on the inactivation of a solution of pepsin by 2.5×10^6 rep. The samples were irradiated in air in stainless steel dishes. The pepsin was irradiated at 10 mg per ml in water and assayed for activity at 0.5 mg per ml. (From Bellamy and Lawton, Nucleonics, **12**, 54–57, 1954.)

accumulated at 1000 rep per min and to high voltage electrons (Hve) accumulated at 143,000 rep per second. For this organism, at least, chain reactions initiated by free radicals do not appear to be involved (9).

It was stated previously that the absorption of energy by ionization is independent of the chemical combination of the atoms. While this may be true, there can be no doubt that the action of the ions formed depends on their chemical structure. This observation has led to the theory that the energy absorbed can migrate down a chain or through a large molecule and break a weak bond. This theory, predicting that certain products should be more common than others as a result of irradiation of large molecules, has led Pollard (8) to refer to energy traps or sinks where breaks occur most frequently. One of the simplest ways of showing that chemical combination affects the end products is to irradiate dry casein and hydrolyzed casein with a few million roentgens. The irradiated dry casein has a mild sweetish odor while the hydrolyzed casein has an unbearable stench!

Isovaleraldehyde, identified as its 2,4-dinitrophenylhydrazone, has been isolated as a product from irradiated leucine. Irradiated dry leucine has very little of the characteristic odor of isovaleraldehyde, but the odor becomes very strong upon the addition of water. This fact, combined with the observation that no ammonia or nitrogen is found by the mass spectrometer until after the addition of water, indicates the following mechanism:



This mechanism seems to indicate a preferred product from the irradiation of leucine and to favor the idea that the energy absorbed in the molecule migrates to form definite products (9).

Just how is all this miscellaneous information related to the effects inside a cell? As has been stated previously, we can only speculate. The following results from experiments on desoxyribonucleoprotein (DNP) illustrate how the conditions of irradiation can magnify the original result by a process not related to chain reactions (15).

The depolymerization of desoxyribonucleic acid (DNA) by ionizing radiation has been studied in some detail (see 16, 17). The depolymerization of DNA or DNP in solution is not a chain reaction, and the ionic yields are much less than one. Therefore, a rather large radiation dose is necessary to produce significant changes in molecular size as measured by changes in viscosity. DNP is much less soluble in physiological (M/6) saline than in lower or higher salt concentrations. It can be made to precipitate as large fibers in the region of minimum solubility. Smaller molecules of DNA are soluble, however, and a condition is set up in which a few breaks in the large nucleoprotein complex will permit rather large fragments of nucleic acid to go into solution. Thus the effect of the irradiation is greatly magnified, and one can conceive that in a living cell the combination of differential solubility and small amounts of radiation magnifies the physiological results of irradiation far beyond the chemical effects.

PART 2. FACTORS MODIFYING SENSITIVITY OF BACTERIA TO IONIZING RADIATIONS[2]

G. E. STAPLETON

The bactericidal effect of radiations has been studied for many years. The extensive literature on radiation microbiology will not be reviewed here; instead we will discuss modern concepts of the mechanism of inactivation of bacteria by ionizing radiations.

Prior to 1948 radiobiologists spent much effort developing a mechanistic theory explaining the lethal effect of ionizing radiations on microorganisms. Most investigators obtained dose-survival curves which suggested that the passage of an ionizing particle through a single macro-

[2] Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

molecule within the cell killed the cell. The following findings indicate the nature of a single direct ionization of a macromolecule: The same fraction of cells was inactivated per unit dose independent of (a) the concentration of cells in an irradiated bacterial suspension, (b) the rate at which the radiation is absorbed, and (c) the temperature, over a moderate range (0–37 C), at which the cells were irradiated. Inactivation showed a dependence on the ion density. On the basis of such evidence Lea (3) postulated that there is only one system in the living cell so delicately poised that elimination of a single unit could result in so drastic an effect, namely, the genetic material. The evidence suggested that death was due to some sort of lethal mutation. The hypothesis just described has served a definite purpose in radiobiological research but is not particularly helpful to those who search for possible antidotes against bactericidal agents.

Recent successes in altering the action of ionizing radiation by changing the environmental conditions have helped us to understand better some of the events which occur between the initial physical process and the physiological or genetic effects observed after irradiation of living cells, particularly bacteria. Treatments utilized for modifying the lethal effects of X- and γ-rays are here divided into pre- and post-irradiation groups. This division is clearly arbitrary; future research may show that sufficient overlapping exists to make such a distinction unwarranted.

Effect of Pretreatment on Bacterial Inactivation

Perhaps the first evidence for a modifying effect of atmospheric conditions on the sensitivity of irradiated microorganisms was that of Anderson and Turkowitz (18) who described a greater radiation resistance for anaerobic or stationary cultures of yeast than for the same organism cultured with vigorous shaking or aeration. They also showed that the effect was related to the oxygen in the irradiated suspension. We have shown the generality of the desensitizing effect of reduction of oxygen concentration in bacterial suspensions during irradiation with X- or γ-rays. The removal of oxygen from the suspension prior to and during irradiation causes a systematic reduction of the slope of the survival curve independent of cultural conditions or the type of organism. The effect is clearly one of dose-reduction, that is, the efficiency of the radiation is reduced by a factor of about 3 by reducing the oxygen concentration in the irradiated bacterial suspension. Several systems which offer protection do so through the same mechanism, namely by reduction of the oxygen concentration of the bacterial suspension prior to and during irradiation. An interesting example is a system in which protection is afforded an irradiated suspension by short-time incubation prior to exposure of X-irradiated *Escherichia coli* in the presence of succinic acid (19). Addition of 0.01 M succinic acid to suspensions incubated at ice-bath temperatures before irradiation produced no detectable protection, whereas incubation at 37 C for 30 min in the presence of this compound afforded striking protection. Respiratory inhibitors, such as cyanide, malonate or iodoacetate, removed the protection afforded by succinate, indicating that oxygen removal by the cells themselves when incubated with an oxidizable substrate prior to irradiation could give protection quantitatively similar to that brought about by removal of oxygen from the suspension by flushing with another gas such as nitrogen. The data on the protective effect of succinate with and without inhibitory systems are shown in figure 3. Further

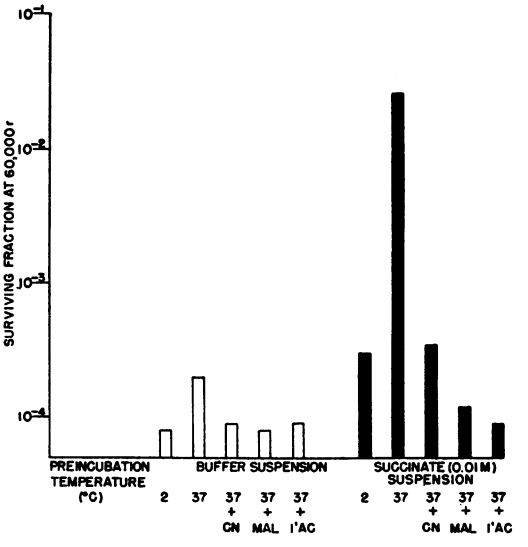


Figure 3. Effect of preincubation with and without respiratory inhibitors on succinate protection against X-ray inactivation of *Escherichia coli*, strain B/r. Survival fraction at an X-ray dose of 60,000 r is shown for the various conditions. □ buffer suspensions, ■ buffered succinate suspensions (0.01 M). Concentration: cyanide and iodoacetate, 0.002 M; malonate, 0.03 M. (Stapleton *et al.*, J. Bacteriol., **63**, 805–811, 1952.)

experiments indicated that hydrogen transfer or enzymatic reduction of compounds within the bacterium at the time of irradiation played an insignificant role in radiation protection in the systems studied (19).

The number of bacteria in the irradiated suspension controls to some extent the number of cells inactivated per unit dose. This effect is accidentally encountered in irradiation of bacterial suspensions containing 10^{11} cells per ml or higher, in equilibrium with air even at ice-bath temperature. This effect is completely removed by saturation of the suspension with oxygen prior to irradiation. The same effect occurs with less concentrated bacterial suspensions at room temperature (20). The increase in survival obtained by using this system is almost identical with that obtained by removing oxygen by other systems and can be explained by removal of oxygen by endogenous respiration of the suspended bacteria before and during irradiation. The published curves for sensitivity of X-irradiated *E. coli* as a function of oxygen concentration of the treated suspension indicate that removal of a few microliters of oxygen per milliliter of suspension in equilibrium with air by endogenous respiration would effect large changes in the survival of the suspension at a constant dose of X-rays.

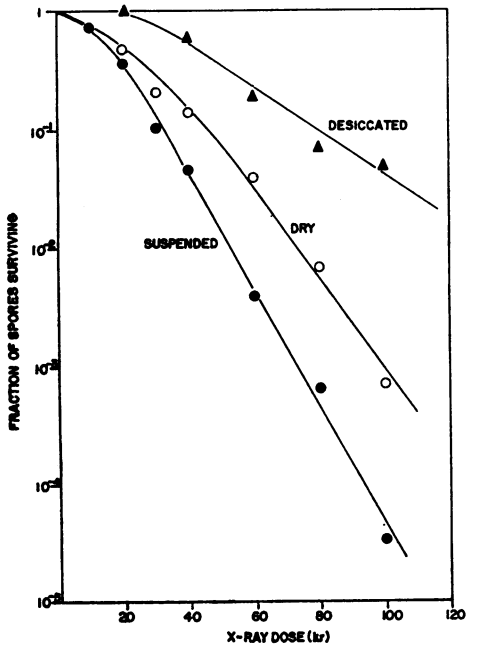


Figure 4. Relation of water content to X-ray sensitivity of *Aspergillus terreus*. (Originally published as figure 2 by Stapleton and Hollaender, J. Cellular Comp. Physiol., Suppl. I, **39**, 101–113, 1952.)

A variety of chemical compounds has been included in bacterial suspensions prior to irradiation in an effort to alter the radiation sensitivity of the cells. A summary of the findings on protection by chemical agents was published by Hollaender and Stapleton (21). The protective action of the chemicals with a few exceptions—notably the sulfhydryl compounds—appeared to be explicable on their ability to lower the oxygen concentration in the bacterial suspensions during irradiations. Among the better chemical protective agents are sodium hydrosulfite and BAL (dimercaptopropanol) (22), compounds known to react with molecular oxygen in solution.

One is led on the basis of the reported findings of the involvement of molecular oxygen in radiation sensitivity of bacteria to the field of radiation chemistry, where several reports have been published concerning the production of H_2O_2 and other oxidizing radicals in aqueous solutions by X- and γ-rays in which oxygen is intimately involved. The probable toxic products of several postulated free radical reactions in X-irradiated aqueous solutions have been proposed by Lea (3), Gray (23), Weiss (24), and others.

It can be argued that radiochemical reactions studied in pure aqueous systems have little if any bearing on the problems of X-ray inactivation of bacteria because the cell fluid is not a pure water system. However, we must keep in mind that water comprises about 75 per cent of the mass of the bacterial cell and that the inactivation reactions probably occur very near the site of an ionization. The role of water and diffusion of toxic materials are evident from two types of experiments. Stapleton and Hollaender (25) as well as others showed that reduction of the water content of spores of the fungus *Aspergillus terreus* reduced the fraction of cells inactivated per unit dose. As shown in figure 4 the efficiency of the radiation decreases with decreasing relative water content. Although the most plausible explanation for the decreased radiation sensitivity of dried spores is the reduction of diffusion of activated molecules, the importance of intracellular water is clearly indicated. Moos (26) has reported similar reduction of X-ray sensitivity

of dried bacteria. The diffusion of activated molecules resulting from irradiation of bacterial suspensions should likewise be reduced by changing the suspension from the liquid to the solid state. Stapleton and Edington (27) showed that reduction of the temperature of suspensions of *E. coli* before and during X-irradiation to liquid nitrogen temperature, about −196 C, resulted in a striking reduction in the fraction of cells inactivated per unit dose as compared with irradiation at ice-bath temperature. An abrupt change in sensitivity occurs at the freezing point of the suspension, several degrees below 0 C. Wood (28) obtained similar results for phage suspensions. At the freezing point of the suspension there is an abrupt change in the coefficient of inactivation. Bacterial suspensions show an oxygen effect at all temperatures investigated. The only plausible explanation yet presented for this is that the important oxygen which enters into the free radical reactions must be trapped close to the sensitive sites within the bacterial cell and that it reacts there even at the low temperature.

We can, I believe, on the basis of the evidence cited, conclude that the radiation inactivation of bacterial suspensions is chiefly a chemical reaction, probably mediated by oxidizing radicals produced in the intracellular water. The effective yield of these reactions can be changed by several systems known to change the ionic yield of oxidizing products of ionization of water, for example, reduction of the oxygen concentration, or by limiting diffusion at temperatures below the freezing point. On the basis of the classical target theory, the over-all effect appears to be a reduction of target diameter.

The literature is full of references to changes in bacterial sensitivity to a wide variety of deleterious agents during the growth cycle; these findings are summarized by Winslow and Walker (29) and others. Usually, "young" bacteria are most sensitive to the action of those chemical and physical agents. Rapidly dividing cells in higher forms of life appear to be the most sensitive to damage by a variety of agents. Reports by Elliker and Frazier (30) and White (31) state that very young bacteria, *i.e.*, bacteria in the lag phase, are more resistant than cells at any other stage of the growth cycle. Our aim has been to correlate the relative sensitivity of *E. coli* with the individual phases of the growth cycle. Mass cultures were made, and at various

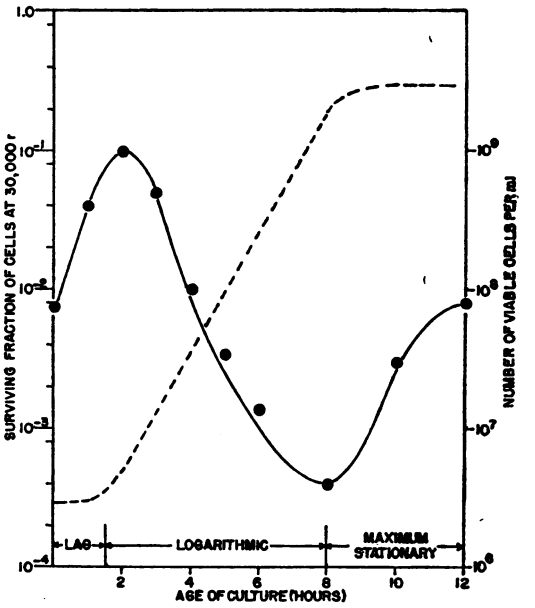


Figure 5. Variation in radiosensitivity of *Escherichia coli* during the growth cycle. (Stapleton, 1952, unpublished thesis.)

intervals of time, aliquots of the growing culture suspension were removed, the cells washed and resuspended in a buffer solution and X-irradiated. Care was taken to adjust the cell concentration in the irradiated suspensions to compensate for growth. Unirradiated cell suspensions were plated along with the irradiated to measure growth as well as to control the experiment. Figure 5 is a graph of the survival of cells at a constant X-ray dose plotted as a function of the age of the culture from the time of inoculation. Phasic changes in sensitivity are shown here which can be correlated with the various phases of the culture cycle. The lag phase is characterized by increased resistance of the bacteria, going through a maximum just before active cell division begins. A rapid decay in resistance occurs during the phase of logarithmic increase in cell numbers, reaching a minimum at the end of this period. As the cells enter the negative acceleration and the maximum stationary phases, there is a gradual return to the initial resistance. The survival curves for cells at the various ages (figure 6) yield a partial explanation for the changes in resistance noted. Although survival curves so far described have been first order with respect to dose of X-rays, those obtained for lag phase cells

are sigmoidal. The multitarget analysis proposed by Atwood and Norman (32) seems especially applicable here since, as shown in figure 6, the sigmoidal curves have almost identical slopes, although the thresholds increase with increasing time in the lag phse. Recent evidence of nuclear changes in bacteria during the lag phase (33, 34) suggests that the peculiarity of survival curves for *E. coli* in the lag phase is evidence that bacterial inactivation is a nuclear process. However, the same type of curves would be expected if the bacteria during the lag phase were in reality multicellular forms rather than multinucleate cells. The correct interpretation depends upon better knowledge of the cytology of this bacterium. The increased sensitivity of cells removed from a growing culture in the logarithmic phase is correlated with an increase in the slope of the survival curves, which can hardly be explained by a change in the number of nuclei per cell. The extreme sensitivity of rapidly dividing cells of higher forms of life appears now to be duplicated in multiplying bacteria. The explanation for this requires further investigation.

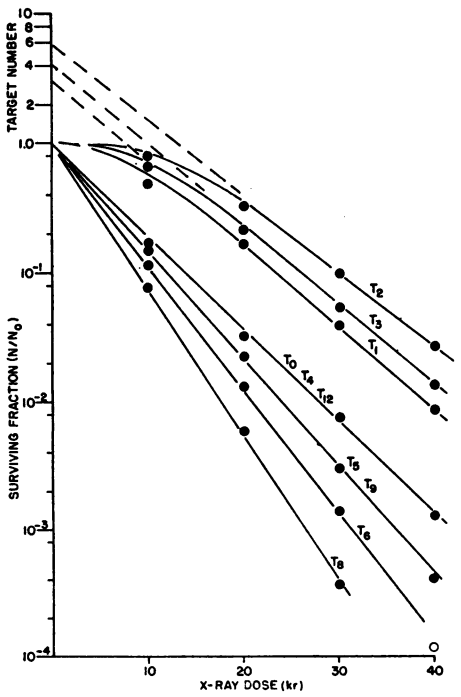


Figure 6. Variation in radiosensitivity of *E. coli* during the growth cycle. The curves labelled T_0, T_1, T_2 . . . T_{12} are survival curves for washed cells removed from the culture at 0 hour, 1 hour, 2 hours, etc., after the time of inoculation. (Stapleton, 1952, unpublished thesis.)

Effect of Some Post Treatments on Bacterial Inactivation

A variety of techniques has been utilized to study reversal or prevention of radiation effects on living cells. Stapleton, Billen, and Hollaender (35) demonstrated partial reversal or alleviation of the effects leading to death of X-irradiated *E. coli* if the cells were incubated at temperatures below those considered optimal for growth of this organism. Billen *et al.* (36, 37) concurrently related reduction of postirradiation incubation temperature with an over-all increase in ability of X-irradiated *E. coli* to oxidize several substrates, as well as a postponement or prevention of radiation-induced release of biologically important compounds. The physiological aberrations investigated by Billen occur only after a latent period of normal metabolic activity. It is perhaps best at this point to describe in some detail the techniques devised for investigating the effect of reduced incubation temperature on bacterial survival after X-irradiation. Aliquots of irradiated and nonirradiated *E. coli* suspensions were plated on nutrient agar equilibrated at various temperatures between 6 and 37 C. The plates were returned to incubators at the various temperatures, and after a 24 hr incubation period all plates were removed to an incubator at 37 C. Colony counts were made after 24 hr at 37 C. Although there was no detectable difference in survival of unirradiated cells with this treatment, the number of viable cells from irradiated suspensions were increased at all temperatures between 12 and 30 C. The surviving fractions of cells at several X-ray doses are plotted in figure 7 as a function of the postirradiation temperature. The plate method was considered to be most appropriate for this type of experiment since the number of colonies on the plates is a direct measure of the number of viable cells under the various conditions.

It is clear that incubation at 18 C is optimal for survival of irradiated cells of this strain of *E. coli*. The rate of recovery was measured by preparing large numbers of plates incubated at the various temperatures. At various intervals of time, plates were removed from the incubators at the different temperatures and rapidly warmed to 37 C until colonies had developed. The data in figure 8 show the results of such an experiment

in which the surviving fraction of cells is plotted as a function of length of incubation at the several temperatures studied. Although the rate of increase in number of viable cells, as measured by the initial slopes of the curves, increases with increasing incubation temperature, the number of viable cells goes through a maximum at 18 C as shown by the data in figure 7. The temperature coefficient for recovery as measured by this method appears to be similar to that for growth of this organism. It is not unlikely that the recovery process involves some synthetic reactions which normally operate during growth of this strain of bacteria. The nature of the curves relating survival of this strain to postirradiation incubation temperature suggests that a synthetic process is involved, the rate of which increases with increasing temperature. A destructive process which has a much higher temperature coefficient sets in at about 18 C and overtakes the synthesis process. The nature of this process is unknown at present but may involve a radiation induced increased thermolability of some vital system(s) in the bacterium. Clark (38) presents some evidence for radiation induced sensitization of proteins to denaturation. Direct measurement of sensitization of *E. coli* protein by radiation is being considered for study at the present time.

It is of interest that the optimal temperature varies for survival of different strains of this species of bacterium. We had previously found large differences in the sensitivity of several strains of *E. coli* as measured by ability to form colonies on nutrient agar at 37 C. Figure 9 shows the survival of three strains of *E. coli* at a constant X-ray dose as a function of postirradiation incubation temperature. It is clear from these data that, although the survival of the three strains is different at 37 C, almost identical survival is indicated at the optimal temperature of each strain. These data indicate that interstrain variations in sensitivity may be dependent on the relative temperature sensitivities of their vital systems. Whether these differences are radiation induced will have to be learned from future physiological experimentation. It seemed desirable to determine whether or not the recovery of bacteria as studied in previous experiments might be an endogenous process, as is the photoreactivation process after ultraviolet ir-

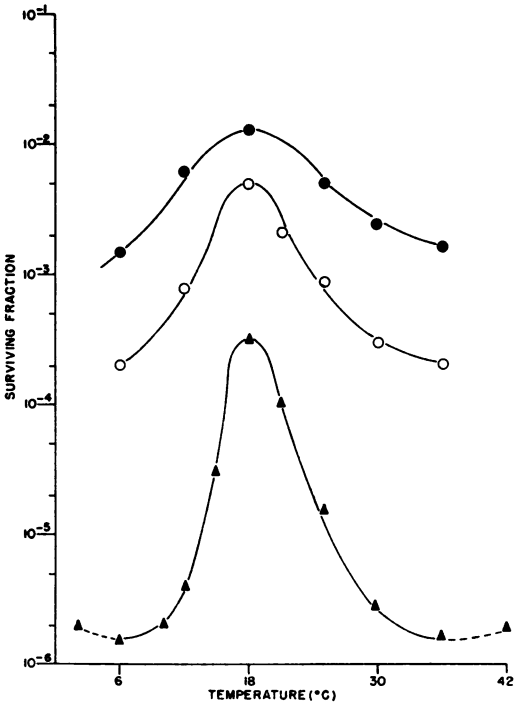


Figure 7. Survival of *E. coli* B/r at several X-ray doses as a function of incubation temperature. ● = 40 kr; ○ = 60 kr; ▲ = 80 kr. (Originally published as figure 1 by Stapleton *et al.*, J. Cellular Comp. Physiol., **41**, 345–358, 1953.)

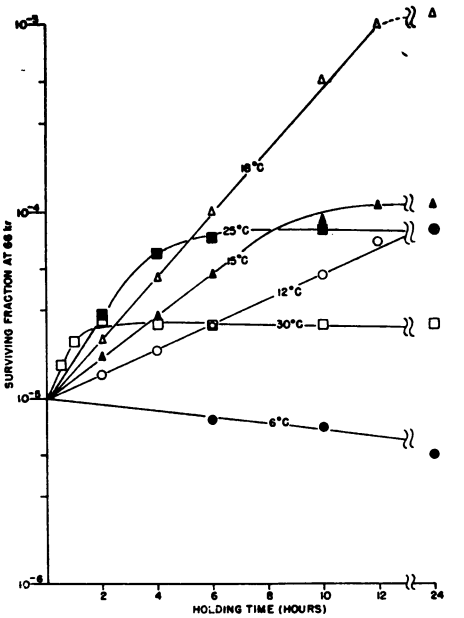


Figure 8. Rates of recovery of *E. coli* B/r at various temperatures after 70 kr of X-rays. (Originally published as figure 2 by Stapleton *et al.*, J. Cellular Comp. Physiol., **41**, 345–358, 1953.)

radiation (39). A synthetic medium was substituted for nutrient broth which had been used in previous experiments. Figure 10 depicts the effect of postirradiation incubation temperature on irradiated *E. coli*, strain B/r, plated on a synthetic medium composed of inorganic salts and glucose and on nutrient broth. The effect of increasing temperature on those cells plated on the synthetic medium is most pronounced. At all temperatures above 6 C there is greater survival on the complex medium, suggesting that some factor or factors contained in the natural materials in nutrient broth stimulate recovery. It is well to note that the synthetic medium will support growth of nonirradiated cells of this strain of bacteria. We have recently attempted to isolate from various extracts of natural materials the factors required for the stimulation of recovery, based on assay at 37 C. Incomplete results indicate that there is a multiple requirement for this process.

In summary, then, we can make a few positive statements concerning the inactivation of some bacteria by ionizing radiations on the basis of the available information: (a) inactivation of aqueous suspensions of cells is predominantly an intracellular, indirect effect, probably mediated by free radical production in the cell fluids; (b) several systems have been found which can modify the efficiency of X- or γ-rays in the production of the lethal effect investigated; (c) the physiological state of the organism at the time of irradiation determines to some extent the sensitivity of the bacterium to radiation; and (d) conditions have been found under which a moderate fraction of a population of irradiated bacterial cells can recover from the damaging effect of ionizing radiations.

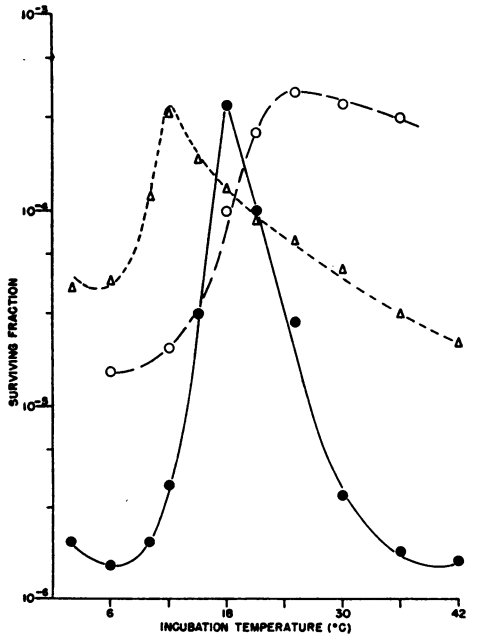


Figure 9. Survival of three strains of *E. coli* at 80 kr at various temperatures. ● = Crooks; ○ = B/r; △ = Texas. (Originally published as figure 4 by Stapleton *et al.*, J. Cellular Comp. Physiol., **41**, 345–358, 1953.)

PART 3. RADIATION INDUCED MUTATIONS AND THEIR IMPLICATIONS ON THE MECHANISMS OF RADIATION EFFECTS ON BACTERIA

MAX R. ZELLE

The past decade has witnessed an astonishing increase in interest and knowledge in the field of bacterial genetics. Progress in radiation biology has likewise been rapid, and the study of the effects of various radiations in bacteria has con-

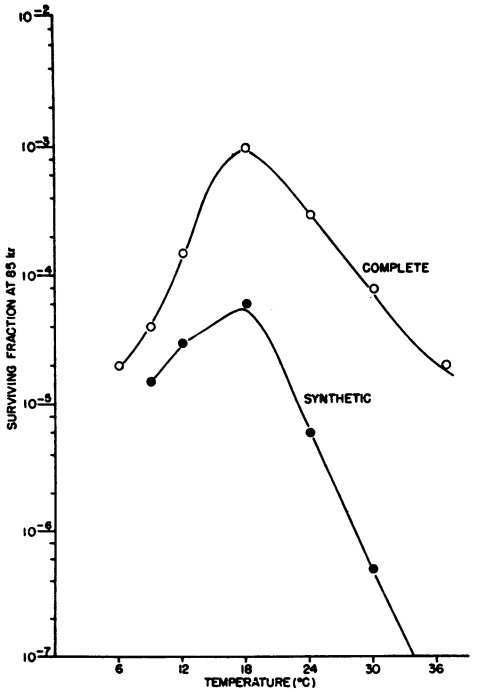


Figure 10. Nutritive requirement for recovery of X-irradiated *E. coli* B/r. (G. E. Stapleton, A. J. Sbarra, A. Hollaender, and D. Billen, in preparation.)

tributed to the development of both fields. It seems unnecessary to consider in any detail the evidence indicating that gene mutation is the fundamental source of heritable variation in bacteria as in higher organisms. This will be assumed to be so in this discussion. The thorough analysis of genetic recombination in *Escherichia coli*, strain K12 (40) essentially proves this assumption.

Prior to this discovery, and in the usual bacterial strain in which genetic recombination is as yet unknown, inferences concerning the genetic mechanism could be based only on analogies in genetic behavior, primarily mutation, in bacteria and in higher organisms amenable to Mendelian analysis. Among these analogies the mutational response to radiations is one of the strongest, and much of the early interest in the induction of mutations in bacteria sprang from this source.

A list of the major radiogenetic analogies might include the following:

(1) All radiations known to be mutagenic in higher organisms are also mutagenic in bacteria.

(2) In general, mutational response increases with increasing radiation dose. However, as will be seen later, the quantitative increase may vary considerably with different mutation systems or different radiations.

(3) In general, the same types or kinds of mutations are induced by radiations as occur spontaneously.

(4) The ultraviolet action spectra for mutation induction in bacteria and in higher organisms are similar with a maximum mutagenic efficiency at about 2600 A corresponding to the absorption maximum of desoxyribonucleic acid (DNA) at this wavelength (41; Zelle and Hollaender, unpublished).

In this paper we will consider only a few phases of the research on radiation induced mutations in bacteria. We will emphasize certain interesting general results and discuss some of the implications of the major findings concerning the mechanisms by which radiations produce inactivation and mutation in the bacterial cell.

Perhaps the chief advantage of bacteria for radiation genetic studies is the relative ease with which specific mutation systems can be studied quantitatively. One of the more interesting general conclusions to emerge from such studies is that different mutation systems may give quite different quantitative results following irradiation.

Delayed Phenotypic Expression

Demerec (42) utilizing the phage spraying technique discovered that all of the irradiation induced mutations to resistance to bacteriophage T1 were not phenotypically expressed until about twelve generations of growth had occurred subsequent to the radiation. Delayed appearance of induced mutations in *E. coli* has also been observed for auxotrophic mutations (43), mutations to streptomycin resistance (44, 45), mutations to nondependence in streptomycin dependent strains (46), and reversions to nondeficiency in various auxotrophic strains (43, 47).

The number of bacterial divisions required for all of the induced mutations to be expressed has been reported to be 2 or 3 for streptomycin resistance (45), about 6 for mutations to independence in several streptomycin dependent strains (46), and was found to vary from about 6 or 7 to about 11 or 12 for different auxotrophic back mutations (47).

The most complete analysis of the factors responsible for the delayed appearance of induced mutations has been made by Newcombe (45) who has shown that the combined delay attributable to (a) segregation of nuclei in multinucleate cells, (b) phenotypic lag (43, 48), (c) delayed mutation, and (d) irregularity in onset of division of the irradiated cells is unlikely ever to exceed seven generations. He attributes the discrepancy observed in mutations to phage resistance largely to a selection artifact inherent in the phage spraying technique which under certain conditions may permit the occurrence of large numbers of spontaneous phage resistant mutants which would be scored as induced mutants.

Ryan (49) has shown that in a histidine dependent strain of *E. coli*, induced histidine independent mutants undergo a variable delay in the onset of division lasting for many generations after nonmutated cells surviving the irradiation have commenced to divide. He postulates that the mutations occur in certain irradiated bacteria which received an injury which delayed the onset of growth and also made mutation likely.

The factors responsible for the delayed appearance of induced mutants are not completely known or understood. It is obvious, however, that experimental precautions which insure that a constant proportion of induced mutations will be expressed must be observed if accurate results

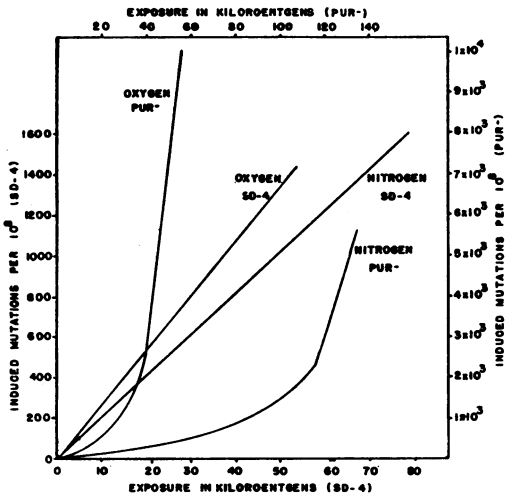


Figure 11. Dose-mutation curves for two mutant strains purineless (pur⁻) and streptomycin dependent (SD-4), both derived from *E. coli* B/r, and irradiated with X-rays in the presence and absence of oxygen (adapted from Anderson, 50).

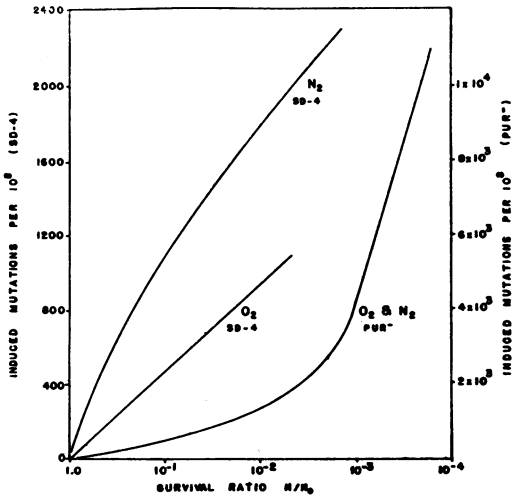


Figure 12. Frequency of induced mutations in derivatives of *E. coli* B/r plotted against survival ratio. Pur⁻: purineless; SD-4: streptomycin dependent (adapted from Anderson, 50).

are to be obtained in quantitative studies of radiation induced mutations in bacteria.

Influence of Oxygen Concentration on X-ray Induced Mutations

Stapleton (this symposium) has discussed the influence of oxygen concentration on the X-ray inactivation of bacteria which, along with other evidence, suggests that inactivation of aqueous suspensions of bacteria is predominantly an intracellular, indirect effect. In studies designed to test if this indirect process had a parallel influence on induced mutations, Anderson (50) found marked differences in the mutagenic response of two different mutants of *E. coli* B/r to X-rays in the presence and absence of oxygen. The mutations studied were to purine independence in the purineless (pur⁻) strain 82/r and to streptomycin independence in the streptomycin dependent strain SD-4. The increase in proportion of induced mutations (figure 11) was a linear function of the X-ray dose for the SD-4 strain and an exponential function for 82/r (pur⁻). A similar difference in mutation-dose curves for these loci has been observed by Zelle and Hollaender (unpublished) for six different wavelengths of ultraviolet (UV) between 2300 and 2967 A.

Figure 11 also shows that, for the pur⁻ strain 82/r, a much greater mutagenic response occurred for a given dose of X-rays when the bacteria were irradiated in oxygen saturated suspensions whereas with the SD-4 back mutation, a very small difference was observed.

When, as in figure 12, the induced mutations were plotted against the survival ratio, it was found that, for a given survival ratio, the SD-4 strain produced about 2 to 2.5 times as many mutations when irradiated in the absence of oxygen, since the organisms had been exposed to about 2.5 times as much radiation. With the pur⁻ strain, identical mutagenic responses were obtained for a given survival ratio regardless of whether the organisms were irradiated in the presence or absence of oxygen. It appears, therefore, that the oxygen-influenced, indirect process which plays a major role in the inactivation of *E. coli* cells and mutation induction in the purineless strain 82/r has very little effect on the induction of mutations to streptomycin nondependence in SD-4.

These same strains were selected by Zelle and Hollaender (unpublished) for monochromatic ultraviolet studies. The action spectra for mutation induction for the two loci were very similar (figure 13) with a maximum of mutagenic efficiency at 2650 A. No statistical significance can be attributed to the irregularity at 2480 A and 2537 A in the SD-4 curve. Similar action spectra with maxima at 2650 A have been reported by Kaplan (41) for three different mutation systems

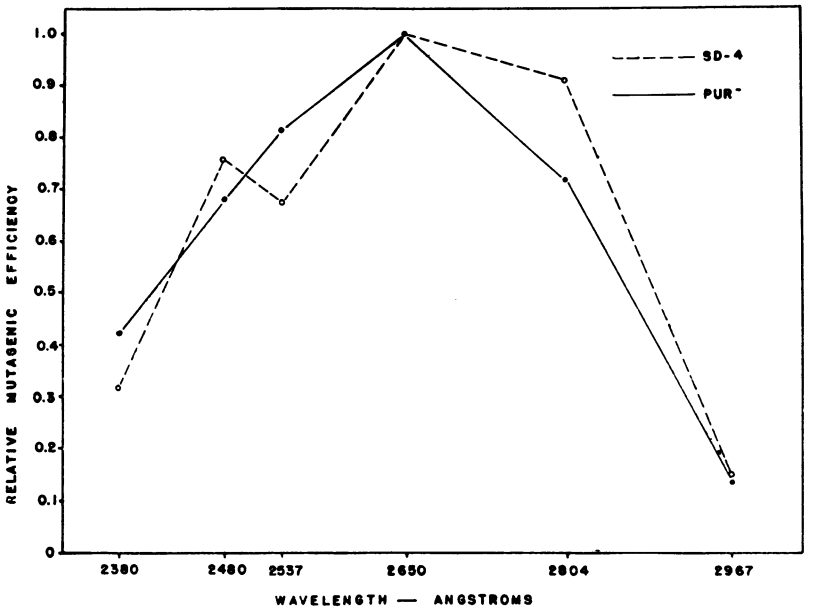


Figure 13. Ultraviolet action spectra for back-mutation in two derivatives of *E. coli* B/r. Dashed line, streptomycin dependence to streptomycin independence; whole line, purine dependence to purine independence (Zelle and Hollaender, unpublished).

in *Serratia marcescens*. In both the SD-4 and 82/r strains, the action spectrum for mutation induction was highly correlated with that for inactivation. Thus these two mutation systems in the same bacterial strain, *E. coli* B/r, which are known to differ markedly in their response to X-rays in the presence and absence of oxygen are very similar in their response to different wavelengths of UV. In both strains, it appears that the chromophores for UV mutation induction and inactivation are identical, probably nucleoprotein.

Photoreactivation and UV Induced Mutations

Kelner (39) was the first to study the effect of photoreactivating light on induced mutations. The most extensive data are those of Newcombe and Whitehead (51) utilizing the color mutations of *E. coli* B/r when plated on mannitol-tetrazolium agar. With this mutation system, a rapid increase in mutations was observed at low UV doses reaching a maximum at about 1000 ergs per mm^2. The proportion of mutants, 12 to 14 per cent, did not change as the UV dose was further increased to 5000 ergs per mm^2 even though the survival ratio continued to decrease. At low UV doses (500 ergs per mm^2) both the lethal and mutagenic effects could be greatly reduced by posttreatment with photoreactivating light whereas, at UV doses greater than 2500 ergs per mm^2, the mutagenic effect becomes stable, and only the lethal effect could be reversed. At low UV doses, the photoreversal was proportionately greater for the mutagenic than for the lethal effect. With the mannitol-tetrazolium color mutants, therefore, the mutagenic and lethal responses to UV and photoreactivating light are not parallel.

In contrast to these results, Novick and Szilard (52) obtained the same proportionate photoreversal in the lethal and mutagenic effects in their studies of three different mutations in B/r to resistance to three of the T series of bacteriophages, T1, T4, and T6. Similarly, Beckhorn (unpublished) obtained identical dose reduction ratios (39) for inactivation and mutation to streptomycin nondependence in the SD-4 streptomycin dependent strain derived from *E. coli* B/r.

Mutation-dose Curves

Figure 14 schematically summarizes the mutation-dose relations for all of the different bacterial mutations adequately investigated thus far. All of these involve specific mutation systems in different mutant derivatives of *E. coli* B/r.

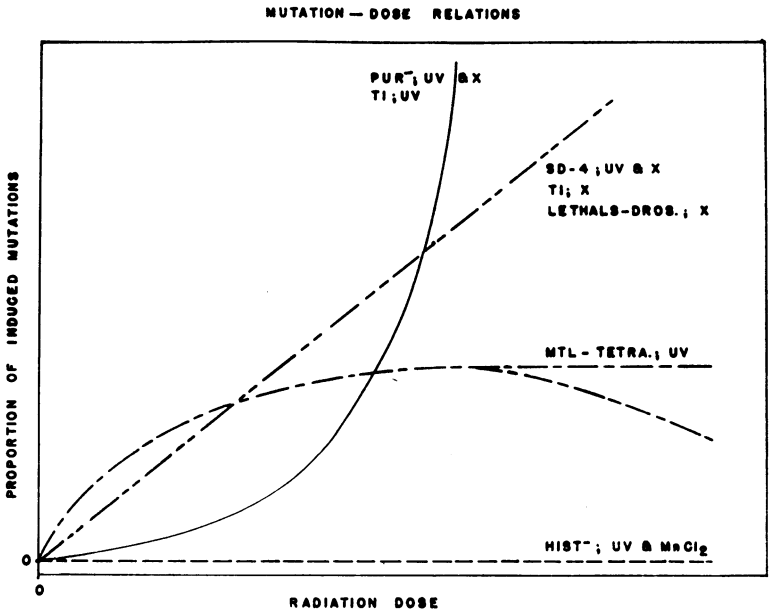


Figure 14. Types of mutation-dose relationships reported for various mutation systems in *E. coli* B/r strains and in *Drosophila melanogaster*.

Mutation systems and strains:

SD-4: mutation to streptomycin nondependence in dependent strain SD-4 (44, 50; Zelle and Hollaender, unpublished).

T_1: mutation to resistance to bacteriophage T_1 in *E. coli* B/r (54).

Pur⁻: mutation to purine independence in purineless strain 82/r (50; Zelle and Hollaender, unpublished).

Mtl-tetra: Mannitol-tetrazolium color mutations in *E. coli* B/r (51).

Hist: mutation to histidine independence (47).

Lethals-Dros: Sex linked lethal mutations in *Drosophila melanogaster* (53).

Mutagenic treatments: UV—ultraviolet; X—X-rays; $MnCl_2$—manganous chloride.

The linear mutation-dose curve indicated for X-ray induced drosophila lethals (Lethals-Dros:X) is perhaps best exemplified by the data of Spencer and Stern (53) for sex linked lethals and may be considered as the classical type of mutation-dose relation. Interpreted within the target theory, the linear relation indicates that a single hit is sufficient to produce a mutation.

Linear mutation-dose relations have been reported in *E. coli* for the SD-4 mutation to streptomycin nondependence following both X-rays (44, 50) and UV (44; Zelle and Hollaender, unpublished), and for T1 phage resistant mutations following X-rays (54). The exponential curve which has already been mentioned for the purineless back mutation in strain 82/r following X-rays (50) and UV (Zelle and Hollaender, unpublished) has also been reported for end point T1 phage resistant mutations after UV irradiation (54). Newcombe and Whitehead (51) found still another type of mutation-dose curve which rises very rapidly at low doses of UV and then reaches a plateau or perhaps passes through a maximum as dosage increases.

Demerec and Cahn (47) discovered two histidineless mutant strains in which no radiation induced back mutations seemed to occur. Even though back mutations of these loci to histidine independence occurred spontaneously, it was not possible to increase the rate of back mutation above the spontaneous level by radiation or other mutagenic treatments.

It seems remarkable that such divergent results should be obtained when so few mutation systems in the same bacterial strain have been adequately tested. Caution should, therefore, be exercised in interpreting mutation dose curves involving mutation at a large number of loci since such curves would appear to be a composite of many different types of mutation response

curves at the individual loci. In this connection, it is realized that mutation at more than one locus may produce the phenotypic change screened for in the study of specific mutational changes. However, the number of loci effecting any particular mutational change is likely very small as compared, for example, to the number of loci in the X chromosome of *Drosophila melanogaster* which can mutate to lethality.

The different types of mutation-dose curves indicate that different mechanisms may be involved in the production of different mutations. The differential mutational response of specific mutation systems to X-rays in the presence and absence of oxygen is a further indication that quite different mechanisms may be involved in the production of different mutations by radiations, and, of course, the occurrence of loci apparently refractive to radiation is of no little interest. Extrapolations to all genetic loci of results obtained with a particular mutation may, therefore, be in error. Hence, the failure to observe, following irradiation, a mutagenic effect with a specific mutation system which parallels the lethal effect does not necessarily prove that lethal mutations are not important in the inactivation of the bacteria.

Mechanism of Radiation Inactivation

When considering the question of the mechanism of radiation damage, it appears to the writer that the interests of clarity are served if, insofar as is possible, the question of the nature of the damage and the mechanisms or processes by which this damage is produced are considered separately.

For many years the effects of radiation were presumed to result from chemical change produced directly in biologically important molecules which absorbed radiation. However, the discoveries of the large influence of oxygen concentration during irradiation, of the influence of postirradiation treatments, and of protection by various chemicals show that a major share of the effects of ionizing radiations occurs by indirect mechanisms.

The effects of UV radiation were similarly believed to result from chemical changes produced by the excitation of the molecules absorbing the UV, and the philosophy behind the action spectrum technique was that a study of the relative effectiveness of different wavelengths would parallel the absorption spectrum of the biologically important molecules and hence furnish evidence concerning the cellular components affected. The phenomenon of photoreactivation coupled with other evidence indicates that a major proportion of the effects of UV is also produced indirectly, and, hence, the action spectrum can only indicate the chromophores or absorbing molecules which initiate the reactions ultimately producing the effect.

With both ionizing and UV radiation, there is a residue of effect which occurs in the absence of oxygen or is not photoreactivable respectively. It has sometimes been assumed that these residues are the direct effects of the radiation, but this is not proven. For example, the effects of X-rays in the absence of oxygen could be produced by direct ionization, could involve a quite different indirect mechanism, or could be produced by the same indirect mechanism which is only accelerated by oxygen.

Turning now to the question of the nature of the lethal change, the inactivation of bacteria by radiation has been considered as due to the production of lethal mutations. Rahn (55) first suggested this hypothesis, and Lea (3) was its strongest proponent. Interpreting all of his data with radiations of different ionization densities, Lea (3) reached the not unreasonable conclusion that *E. coli* possessed 250 genes, each of an average diameter of 12 millimicrons, capable of mutating to lethality.

The discovery of indirect mechanisms of production of radiation effects forces a revision of the target theory and a reinterpretation of action spectra but does not necessarily force the abandonment of the lethal mutation hypothesis. It is known that some mutations are produced by these indirect mechanisms and, hence, that lethal mutations could be.

There is, however, a growing body of evidence which casts doubt on the lethal mutation hypothesis. Consequently, a brief examination of some of the experimental facts bearing on the question seems desirable.

Observations Bearing on the Lethal Mutation Hypothesis

What are the principal observations forming the foundation of the lethal mutation hypothesis?

With ionizing radiations, first order kinetics *i.e.*, exponential killing, is generally observed. If significance is attached to the kinetics observed

(see Heinmets *et al.*, 56, for a contrary view), the exponential survival curve indicates that a unitary action or single event produced the effect. Other data, *i.e.*, independence of intensity and increasing dose required for inactivation with increasing ionization density, indicate the unit event to be a single ionization. The consequence of a single ionization is chemical change in the ionized molecule. The lethal effect, therefore, appears to result from chemical change in a single molecule. As Rahn (55) pointed out, the gene is a most logical candidate for the kind of molecule so vital to the cell that a change in a single one could be lethal to the cell.

With UV, the kinetic picture is not as clear, but in many cases bona fide exponential as well as sigmoidal or multihit curves have been observed. *E. coli*, strains B and B/r, giving exponential and sigmoidal killing curves respectively are a case in point (57). The occurrence of exponential killing in some bacteria with UV likewise suggests a unitary action, and a similar argument likewise leads to the gene. This concept is supported by the commonly observed 2650 A maximum in efficiency of UV killing and for mutation induction (41, Zelle and Hollaender, unpublished) which suggests absorption of UV quanta in nucleoprotein molecules as the initial step in both inactivation and mutation induction.

It would appear that lethal and visible mutations would show a close parallel behavior in radiation experiments, and, hence, that a high correlation should be observed between the bactericidal and mutagenic effects of radiation. The similarity in mutation and inactivation UV action spectra, the approximately equal reversal of effect by photoreactivating light, and the equal X-ray-oxygen effect upon at least one mutation system are examples of such correlations.

Lethality and mutation are not always correlated, however. Demerec and Latarjet (54) observed that for a given dose of UV, the same proportions of induced T1 phage resistant mutations are observed in *E. coli*, strains B and B/r, whereas the bactericidal effect is much greater in strain B. Their data are scanty, however, and Witkin (58) presents data which show about 2 to 2.5 times as high a frequency of lactose negative mutations in strain B/r than in strain B at the same UV dose. Although this does not strictly parallel the difference in lethal effect, it does indicate that equal mutagenic effects are not always obtained following irradiation of these two strains.

The failure of Anderson (50) to obtain correlated lethal and mutagenic effects in the SD-4 mutation to streptomycin nondependence following X-rays in the presence and absence of oxygen has already been discussed. Similarly, Newcombe and Whitehead (51) found several differences in quantitative response between induced mannitol-tetrazolium color mutants and inactivation following UV irradiation and exposure to photoreactivating light.

The frequency of induced mutations and the survival ratios following UV irradiation was reduced by postirradiation incubation at 15 C as compared to 37 C (59). This observation is incompatible with the lethal mutation hypothesis, for on this basis higher survival ratios would be expected at 15 C than at 37 C since fewer lethal mutations would be induced. More extensive data on the frequency of X-ray induced mutations and survival at different postirradiation temperatures have been obtained by Billen and Whittle (60) who used four different auxotrophic strains and six different temperatures ranging from 6 to 37 C. The effects of temperature treatment on mutation and inactivation were not correlated although with two strains roughly parallel responses were obtained. The four strains varied both quantitatively and qualitatively in their mutational and lethal responses to the X-rays at the different temperatures.

Other instances of nonparallel lethal and mutagenic responses to radiation have been published. These certainly do not support the hypothesis of lethal mutations as the ultimate bactericidal damage. As pointed out earlier, however, because of the rather wide differences in response of specific mutations to radiation, it would seem unwarranted to assume that lethal mutations would behave exactly like any particular visible mutation.

Lederberg *et al.* (40) and Beckhorn (61) have independently approached the question of recessive lethal mutations somewhat more directly by studies involving radiation of semipermanent diploid *E. coli* K12 cells. The majority of the immediate progeny of the diploid cells surviving the irradiation was found to be haploid, not diploid. Both X-rays and UV produced this haploidization effect, and Beckhorn (61) showed the UV effect to be photoreversible. If recessive lethal formation were an important factor in killing,

formation of haploid cells would be prevented since, in the haploids, recessive lethals would be expressed whereas in a diploid they would be masked.

Witkin (58) found a correlation between the average number of nuclei per cell and lactose negative mutant sector formation in *E. coli*, strains B and B/r, which indicates that sector formation is at least partially due to nuclear segregation in multinucleate cells. As she points out, with survival ratios as low as 10^{-3} as in her experiments, the chance of any particular cell having more than one nucleus is very low. Hence no correlation would be expected if killing were due to lethal mutations or, indeed, if killing were nuclear at all. Witkin adds the further observation that resistance to UV does not parallel changes in the average number of nuclei per cell at different growth stages.

In similar studies of lactose negative mutation in *E. coli* B/r, Newcombe (45) did not observe an increase in the proportion of whole colony mutants with increasing dose as would be expected if killing were nuclear since the relative proportion of cells with more than one viable nucleus would be markedly decreased at higher doses.

Summarizing, the foregoing observations indicate that radiation inactivation of *E. coli* B/r cannot be due primarily to recessive lethal mutations and may not involve nuclear inactivation at all. There are, however, other data in addition to those already mentioned in support of the lethal mutation hypothesis which indicate that the lethal effects of radiation may be the result of nuclear inactivation.

Atwood and Norman (32) have elaborated an interpretation of sigmoidal survival curves which holds that such sigmoidal curves result when more than one target must be inactivated to produce killing and each target is inactivated by a single hit. The number of targets can be estimated by extrapolation of the linear portion of the semilogarithmically plotted survival curve back to zero dose. Neurospora conidia furnish unique material to test this interpretation and to analyze genetically the nature of the damage resulting in inactivation. Atwood and Mukai (62) and Norman (63) have utilized X-rays and UV respectively in such studies. Space does not permit detailed consideration of their results beyond the following general conclusions: (a) recessive lethals are induced by radiation; (b) they are important in inactivation of uninucleate conidia but are of relatively little importance in multinucleate conidia; (c) inactivation of conidia is a function of inactivation of nuclei; (d) nuclei are inactivated with first order kinetics.

Norman (63) postulates that in addition to recessive lethal mutations, nuclei are inactivated by a nongenetic damage of such a nature that if a nucleus sustaining such injury did succeed in dividing, the injury would not be inherited by the daughter nuclei. His results show that the UV quantum is sufficiently energetic to cause this nongenetic nuclear inactivation, that only quanta absorbed in the nucleus are able to cause inactivation by either recessive lethal mutations or by the nongenetic damage, and that both types of injury are reduced by photoreactivation. The UV action spectra for both recessive lethal mutation induction and the nongenetic effect are similar to the absorption spectrum of nucleic acid and to the action spectra for the lethal and mutagenic effects in bacteria.

Since cytological and genetic evidence indicates that *E. coli* and other rod bacteria are multinucleate at certain growth stages, a parallel biological situation exists in certain bacteria. Atwood and Stapleton (64) have pointed out that sigmoidal survival curves would be observed in a multinucleate bacterial species on either the recessive lethal or dominant lethal hypotheses. In addition, they show that exponential X-ray-survival curves could occur in multinucleate species if a type of dominant lethal mutation is postulated which, when present in one nucleus, precludes division of the cell or of daughter cells, or if a single ionizing electron is capable of causing lethal damage in all of the nuclei present. That the latter alternative may actually be so is indicated by their observation of an exponential survival curve with *E. coli* B/r cells irradiated with 250 kilovolt peak (KVP) X-rays but a sigmoidal curve extrapolating to a target number of about two (32) with less densely ionizing 1.1 Mev γ-rays from Co^{60}.

Stapleton (this symposium) has discussed the changes in resistance to X-rays and the shape of the survival curves of *E. coli* B/r cells at different stages of the growth cycle. Analysis by the multitarget hypothesis leads to estimates of target numbers which correspond well with the nuclear number per cell. He points out, however, that the presumed multinucleate cells may actually be multicellular forms. In studies of the

survival following UV of *Aerobacter aerogenes* cells on different energy substrates, Norman (65) found a close correspondence between the extrapolated target number per bacterium and the average number of nuclei per cell as determined cytologically. The particular energy substrate influenced the ultimate slope of the survival curves but did not influence the estimate of target number.

Stapleton's (this symposium) and Norman's (65) findings seem at odds with Witkin's (58) observation of a lack of correlation between UV resistance and nuclear number. They are compatible with the concept that bacterial inactivation by radiation is largely nuclear in their experiments and thus are at direct odds with the very critical evidence supplied by the nuclear number-mutant sector correlations found by Witkin (58).

Obviously, the question cannot be resolved on the basis of present information. In view of the fact that inactivation has been shown conclusively to be a function of nuclear inactivation in neurospora conidia and that the degree of ploidy in yeasts is correlated with radiation resistance and the kinetics observed (66, 67), the writer is reluctant to conclude that inactivation of bacterial cells by radiation is largely independent of the nucleus.

The data can be reconciled if it is assumed that the inactivation is largely the result of a type of nongenetic nuclear damage similar to that postulated by Norman (63) and that a damaged nucleus may recover if the cell contains an undamaged nucleus. Norman (63) cites some unpublished data of Atwood's which indicate that such recovery may occur in multinucleate, heterokaryotic neurospora conidia.

Suppose, however, that it is concluded that radiation inactivation of bacteria is nonnuclear. What other kinds of injury can be postulated as the ultimately lethal damage?

Dale (68) has suggested enzyme inactivation, but even though certain enzymes are quite sensitive to radiation, it is difficult to account for the first order kinetics frequently observed since it is unlikely that inactivation of a single enzyme molecule among many such molecules would be lethal to the cell. McIlwain (69) has suggested that certain enzymes may be present in only one or a few copies. With such an enzyme, the first order kinetics could be explained. If, as McIlwain (70) has also suggested, the genes rather than controlling the specificity or synthesis of such an enzyme actually functioned as the enzyme, the enzymatic and lethal mutation hypotheses merge.

It has been postulated by Kelner (71) that the basic effect of UV on a bacterial cell is the immediate and specific inhibition of DNA synthesis and that many of the other effects of UV such as lethality, mutation induction, and inhibition of adaptive enzyme synthesis may be secondary consequences of this fundamental effect. Newcombe (45) has made a similar suggestion that the basic effect of irradiation may be an inhibition or change in end products of a number of vital syntheses of which gene duplication is only one. These inhibitions, presumably, would lead to the death of the cell. It is not possible to separate cause from effect in the present state of knowledge, but if any significance is attached to the first order or low order kinetics almost always observed, one or a few UV quanta or X-ray ionizations can produce the effect. The simplest mechanism whereby a UV quantum or X-ray ionization could inhibit or alter a number of cellular syntheses would appear to be by inactivation of the nucleus.

It is realized that postulating nuclear inactivation as the basic lethal damage caused by radiations does little towards specifying the exact nature of the damage, but if such a conclusion could be made, it would facilitate a more rational attack on the problem in subsequent investigations.

One further point seems worthwhile. Luria (72) was the first to suggest from microscopic examination of the behavior of irradiated bacteria that killing seemed to occur in more than one manner. Zelle and Hollaender (73) and Heinmets *et al.* (56) have also put forward this view. In this connection, the induction of bacteriophage production and lysis in lysogenic bacteria by small doses of radiation is a quite different bactericidal effect of radiation (74).

A comparison of *E. coli*, strain B, and its radiation resistant mutant, strain B/r, is interesting from the viewpoint of multiple mechanisms and effects. Presumably, these two organisms differ by a single mutation (57) and thus are as identical genetically as it is possible to be and still be different.

Strain B is extremely radiation sensitive, more sensitive than any other strain of *E. coli* known.

Strain B/r is significantly more resistant than B although it seems about average when compared to a number of other *E. coli* strains (Stapleton, unpublished; see also table 10-2, Zelle and Hollaender, 73). Several markedly more resistant strains are known.

Strain B forms filamentous cells following small doses of UV; strain B/r does not (57). No filamentous cells are formed after X-rays (75). Even though these filamentous cells appear to possess many nuclei, they have no greater resistance to radiation than normal B cells (57). The filamentous cells also yield the highest ratio of intact to sectored lactose negative mutant colonies (58). Observations on these filamentous, strain B cells, therefore, contribute only further confusion to the question of nuclear versus some other form of lethal damage or, for that matter, on the question of a genetic function of the presumed nuclei.

Strain B is killed exponentially by UV whereas strain B/r produces a sigmoidal survival curve (57). Both strains are killed exponentially by X-rays (54). Roberts and Aldous (75) have shown that B partially recovers from UV when held in liquid media. B/r exhibits no such recovery nor does B following X-radiation. B shows significant heat reactivation (76) but does not exhibit appreciable catalase reactivation following UV (77) whereas the inverse is true for strain B/r.

Thus the observations on these two closely related strains indicate that a number of different mechanisms exist whereby radiations produce lethal effects and that more than one kind of lethal damage is produced.

It appears likely that a number of mechanisms producing a number of different lethal effects will be found which may vary in relative importance in different bacterial strains. Caution should, therefore, be exercised in making generalizations concerning the lethal effects as well as the genetic effects of radiations in bacteria. At first glance, this seems to be a pesssimistic conclusion. However, the fact that such caution is indicated is actually evidence of rapid progress in the past few years, for it is now evident that the interpretations of about ten years ago were much too simple. Progress should continue to be rapid in the immediate future, for more critical physical and biological techniques can be employed in the analysis now than ever before.

REFERENCES

1. Lawton, E. J., Bellamy, W. D., Hungate, R. E., Byrant, M. P., and Hall, E. 1951 Some effects of high velocity electrons on wood. Science, **113**, 380–382.
2. Lawton, E. J., Bellamy, W. D., Hungate, R. E., Bryant, M. P., and Hall, E. 1951 Studies on the changes produced in wood exposed to high velocity electrons. Tappi, **34**, 113A–116A.
3. Lea, D. E. 1947 *Actions of radiations on living cells*. The MacMillan Co., New York, N. Y.
4. Morrison, P. 1952 Radiation in living matter: The physical processes. In Nickson, J. J., *Symposium on radiobiology*. John Wiley, New York, N. Y.
5. Fano, U. 1952 Secondary electrons: Average energy loss for ionization. In Nickson, J. J., *Symposium on radiobiology*. John Wiley, New York, N. Y.
6. Allen, A. O. 1952 Mechanism of decomposition of water by ionizing radiation Discussions Faraday Soc., No. 12 *Radiation chemistry*, pp. 79–87.
7. Bonet-Maury, P. 1952 Chemical phenomena in irradiated pure water. Discussions Faraday Soc., No. 12 *Radiation chemistry*, pp. 72–79.
8. Pollard, E. C. 1953 *The physics of viruses*. Academic Press, New York, N. Y.
9. Bellamy, W. D., and Lawton, E. J. 1954 Evaluation of some of the problems related to sterilization by high voltage electrons. Nucleonics, **12**, 54–57.
10. Dale, W. M. 1952 The indirect action of ionizing radiations on aqueous solutions and its dependence on the chemical structure of the substrate. J. Cellular Comp. Physiol., **39**, Suppl. I, 39–55.
11. McDonald, M. 1950 Organization of the chromosome. Carnegie Inst. Wash. Publ., No. 49, 173–177.
12. Schmitz, J. V., and Lawton, E. J. 1951 Initiation of vinyl polymerization by means of high-energy electrons. Science, **113**, 718–719.
13. Mead, J. F. 1952 The irradiation-induced autoxidation of linoleic acid. Science, **115**, 470–472.
14. Hannan, R. S., and Shepherd, H. J. 1952 An after effect in butter-fat irradiated with high-energy electrons. Nature, **170**, 1021–1022.
15. Rozendaal, H. M., Bellamy, W. D., and Baldwin, T. H. 1951 Effects of ionizing

radiations upon isolated deoxyribosenucleoprotein fibres. Nature, **168,** 694.

16. TAYLOR, B., GREENSTEIN, J. P., AND HOLLAENDER, A. 1947 The action of X-rays on thymus nucleic acid. Cold Spring Harbor Symposia Quant. Biol., **12,** 237–246.
17. BUTLER, J. A. V., AND SMITH, K. A. 1950 Degradation of deoxyribonucleic acid by free radicals. Nature, **165,** 847–848.
18. ANDERSON, R. S., AND TURKOWITZ, H. 1941 The experimental modification of the sensitivity of yeast to roentgen rays. Am. J. Roentgenol. Radium Therapy Nuclear Med., **46,** 537–542.
19. STAPLETON, G. E., BILLEN, D., AND HOLLAENDER, A. 1952 The role of enzymatic oxygen removal in chemical protection against X-ray inactivation of bacteria. J. Bacteriol., **63,** 805–811.
20. HOLLAENDER, A., AND STAPLETON, G. E. 1953 New aspects of the oxygen concentration effect in X-ray inactivation of bacterial suspensions. Federation Proc., **12,** 70.
21. HOLLAENDER, A., AND STAPLETON, G. E. 1953 Fundamental aspects of radiation protection from a microbiological point of view. Physiol. Revs., **33,** 77–84.
22. BURNETT, W. T., JR., STAPLETON, G. E., MORSE, M. L., AND HOLLAENDER, A. 1951 Reduction of X-ray sensitivity of *Escherichia coli* B/r by sulfhydryl compounds, alcohols, glycols, and sodium hydrosulfite. Proc. Soc. Exptl. Biol. Med., **77,** 636–638.
23. GRAY, L. H. 1947 Quoted by LEA, D. E., in *Actions of radiations on living cells*, p. 67. The MacMillan Co., New York, N. Y.
24. WEISS, J. 1947 Some aspects of the action of radiations on aqueous solutions. Brit. J. Radiol. (Suppl. I), 56–58.
25. STAPLETON, G. E., AND HOLLAENDER, A. 1952 Mechanism of lethal and mutagenic action of ionizing radiations on *Aspergillus terreus*. II. Use of modifying agents and conditions. J. Cellular Comp. Physiol., **39** (Suppl. I), 101–113.
26. Moos, W. S. 1952 Variation of irradiation effects on microorganisms in relation to physical changes of their environment. J. Bacteriol., **63,** 688–690.
27. STAPLETON, G. E., AND EDINGTON, C. W. 1953 Temperature dependence of bacterial inactivation by X-rays. J. Radiation Research (Abstract), **1,** 229–230.
28. WOOD, T. H. 1954 Dependence of X-ray sensitivity of bacteriophage on phase state and temperature. Nature, **173,** 641–642.
29. WINSLOW, C.-E. A., AND WALKER, H. H. 1939 The earlier phases of the bacterial culture cycle. Bacteriol. Revs., **3,** 147–186.
30. ELLIKER, P. R., AND FRAZIER, W. C. 1938 Influence of time and temperature of incubation on heat resistance of *Escherichia coli*. J. Bacteriol., **36,** 83–98.
31. WHITE, H. R. 1952 Heat disinfection of *Streptococcus faecalis* and *Streptococcus lactis*. Proc. Soc. Appl. Bacteriol., **15,** 8–14.
32. ATWOOD, K. C., AND NORMAN, A. 1949 On the interpretation of multi-hit survival curves. Proc. Natl. Acad. Sci. U. S., **35,** 696–709.
33. ROBINOW, C. F. 1945 Nuclear apparatus and cell structure of rod-shaped bacteria. *Addendum* in *The bacterial cell*, pp. 355–377, by Dubos, R. J. Harvard University Press, Cambridge, Mass.
34. DELAMATER, E. D., AND MUDD, S. 1951 The occurrence of mitosis in the vegetative phase of *Bacillus megatherium*. Exptl. Cell Research, **2,** 499–512.
35. STAPLETON, G. E., BILLEN, D., AND HOLLAENDER, A. 1953 Recovery of X-irradiated bacteria at suboptimal incubation temperature. J. Cellular Comp. Physiol., **41,** 345–358.
36. BILLEN, D., STAPLETON, G. E., AND HOLLAENDER, A. 1953 The effect of X-radiation on the respiration of *Escherichia coli*. J. Bacteriol., **65,** 131–135.
37. BILLEN, D., STREHLER, B. L., STAPLETON, G. E., AND BRIGHAM, E. 1953 Postirradiation release of adenosinetriphosphate from *Escherichia coli* B/r. Arch. Biochem. and Biophys., **43,** 1–10.
38. CLARK, J. H. 1938 The temperature coefficient of the effect of radiation on proteins and its relation to injury of the living cell. Am. J. Roentgenol. Radium Therapy Nuclear Med., **40,** 501–508.
39. KELNER, A. 1949 Photoreactivation of ultraviolet-irradiated *Escherichia coli*, with special reference to the dose-reduction principle and to ultraviolet-induced mutation. J. Bacteriol., **58,** 511–522.
40. LEDERBERG, J., LEDERBERG, E. M., ZINDER, N. D., AND LIVELY, E. R. 1951 Recombination analysis of bacterial heredity. Cold Spring Harbor Symposia Quant. Biol., **16,** 413–441.
41. KAPLAN, R. W. 1952 Auslösung von Farbsektor und anderen Mutationen bei *Bacterium prodigiosum* durch monochromatisches Ultraviolett verschiedener Wellenlängen. Z. Naturforsch., **7b,** 291–304.
42. DEMEREC, M. 1946 Induced mutations and

possible mechanisms of the transmission of heredity in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S., **32**, 36–46.

43. DAVIS, B. D. 1950 Studies on nutritionally deficient bacterial mutants isolated by means of penicillin. Experientia, **6**, 41–50.
44. DEMEREC, M. 1951 Studies of the streptomycin-resistance system of mutations in *E. coli*. Genetics, **36**, 585–597.
45. NEWCOMBE, H. B. 1953 The delayed appearance of radiation-induced genetic change in bacteria. Genetics, **38**, 134–151.
46. LABRUM, E. L. 1953 A study of mutability in streptomycin-dependent strains of *Escherichia coli*. Proc. Natl. Acad. Sci. U. S., **39**, 280–288.
47. DEMEREC, M., AND CAHN, E. 1953 Studies of mutability in nutritionally deficient strains of *Escherichia coli*. I. Genetic analysis of five auxotrophic strains. J. Bacteriol., **65**, 27–36.
48. NEWCOMBE, H. B. 1948 Delayed phenotypic expression of spontaneous mutations in *Escherichia coli*. Genetics, **33**, 447–476.
49. RYAN, F. J. 1954 The delayed appearance of mutants in bacterial cultures. Proc. Natl. Acad. Sci. U. S., **40**, 178–186.
50. ANDERSON, E. H. 1951 The effect of oxygen on mutation induction by X-rays. Proc. Natl. Acad. Sci. U. S., **37**, 340–349.
51. NEWCOMBE, H. B., AND WHITEHEAD, H. A. 1951 Photoreversal of ultraviolet-induced mutagenic and lethal effects in *Escherichia coli*. J. Bacteriol., **61**, 243–251.
52. NOVICK, A., AND SZILARD, L. 1949 Experiments on light reactivation of ultraviolet inactivated bacteria. Proc. Natl. Acad. Sci. U. S., **35**, 591–600.
53. SPENCER, W. P., AND STERN, C. 1948 Experiments to test the validity of the linear r dose/mutation frequency relation in *Drosophila* at low dosage. Genetics, **33**, 43–74.
54. DEMEREC, M., AND LATARJET, R. 1946 Mutations in bacteria induced by radiations. Cold Spring Harbor Symposia Quant. Biol., **11**, 38–49.
55. RAHN, O. 1930 The order of death of organisms larger than bacteria. J. Gen. Physiol., **14**, 315–337.
56. HEINMETS, F., LEHMAN, J. J., TAYLOR, W. W., AND KATHAN, R. H. 1954 The study of factors which influence metabolic reactivation of the ultraviolet inactivated *Escherichia coii*. J. Bacteriol., **67**, 511–522.
57. WITKIN, E. M. 1947 Genetics of resistance to radiation in *Escherichia coli*. Genetics, **32**, 221–248.
58. WITKIN, E. M. 1951 Nuclear segregation and the delayed appearance of induced mutants in *Escherichia coli*. Cold Spring Harbor Symposia Quant. Biol., **16**, 357–371.
59. BERRIE, A. M. M. 1953 The effects of temperature on ultraviolet-induced mutability in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S., **39**, 1125–1133.
60. BILLEN, D., AND WHITTLE, R. 1953 Effects of postirradiation temperature treatment on induced mutations in *Escherichia coli*. Biology Division, Oak Ridge Natl. Lab., Semiannual progress report for period ending August 15, 1953, pp. 38–40.
61. BECKHORN, E. J. 1950 The effect of ultraviolet radiation on a heterozygous strain of *Escherichia coli* and some of its segregants. Ph.D. Thesis, Cornell University.
62. ATWOOD, K. C., AND MUKAI, F. 1951 Radiation effects on *Neurospora* conidia. Biology Division, Oak Ridge Natl. Lab., Quarterly progress report for the period ending November 10, 1951, pp. 29–33.
63. NORMAN, A. 1951 Inactivation of *Neurospora* conidia by ultraviolet radiation. Exptl. Cell Research, **2**, 454–473.
64. ATWOOD, K. C., AND STAPLETON, G. E. 1952 Lethal mutations and the bactericidal action of ionizing radiation. Naturwissenschaften, **39**, 330–331.
65. NORMAN, A. 1953 Production of phenocopies in *Aerobacter aerogenes* by ultraviolet radiation. J. Bacteriol., **65**, 151–156.
66. LATARJET, R., AND EPHRUSSI, B. 1949 Courbes de survie de levures haploides et diploides soumises aux rayons X. Compt. rend., **229**, 306–308.
67. ZIRKLE, R. E., AND TOBIAS, C. A. 1953 Effects of ploidy and linear energy transfer on radiobiological survival curves. Arch. Biochem. and Biophys., **47**, 282–306.
68. DALE, W. M. 1940 The effect of X rays on enzymes. Biochem. J., **34**, 1367–1373.
69. MCILWAIN, H. 1946 The magnitude of microbial reactions involving vitamin-like compounds. Nature, **158**, 898–902.
70. MCILWAIN, H. 1947 Interrelations in microorganisms between growth and the metabolism of vitamin-like substances. Advances in Enzymol., **7**, 409–460.
71. KELNER, A. 1953 Growth, respiration, and nucleic acid synthesis in ultraviolet-irradiated and in photoreactivated *Escherichia coli*. J. Bacteriol., **65**, 252–262.
72. LURIA, S. E. 1939 Action des radiations sur le *Bactérium coli*. Compt. rend., **209**, 604–606.

73. ZELLE, M. R., AND HOLLAENDER, A. 1954 Effects of radiation on bacteria. In *Radiation biology. Vol. 2*, chapter 10. Edited by Hollaender, A. McGraw Hill Book Co., New York, N. Y.

74. LWOFF, A., SIMINOVITCH, L., KJELDGAARD, N. A. 1950 Induction de la production de bactériophages chez une bactérie lysogène. Ann. inst. Pasteur, **79**, 815–859.

75. ROBERTS, R. B., AND ALDOUS, E. 1949 Recovery from ultraviolet irradiation in *Escherichia coli*. J. Bacteriol., **57**, 363–375.

76. ANDERSON, E. H. 1951 Heat reactivation of ultraviolet-inactivated bacteria. J. Bacteriol., **61**, 389–394.

77. LATARJET, R., AND CALDAS, L. R. 1952 Restoration induced by catalase in irradiated microorganisms. J. Gen. Physiol., **35**, 455–470.